BTY 201 MOLECULAR BIOLOGY

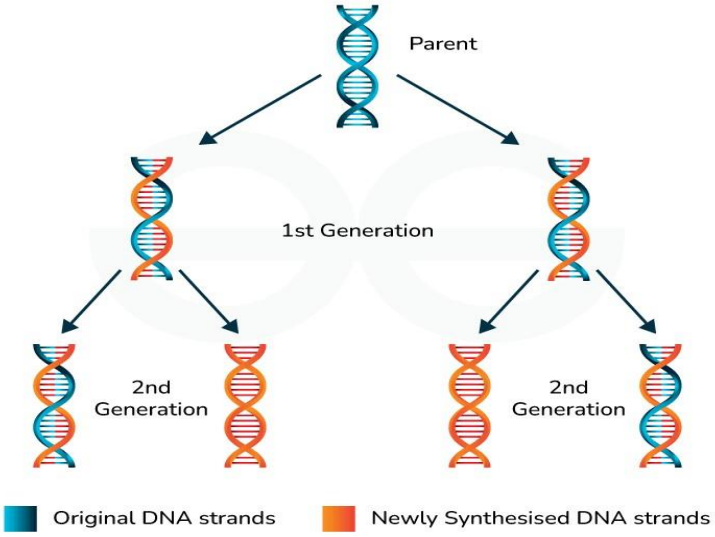
UNIT 1

MODELS OF DNA REPLICATION :

The dispersive, semi-conservative, and conservative models are three distinct approaches to depict DNA replication.

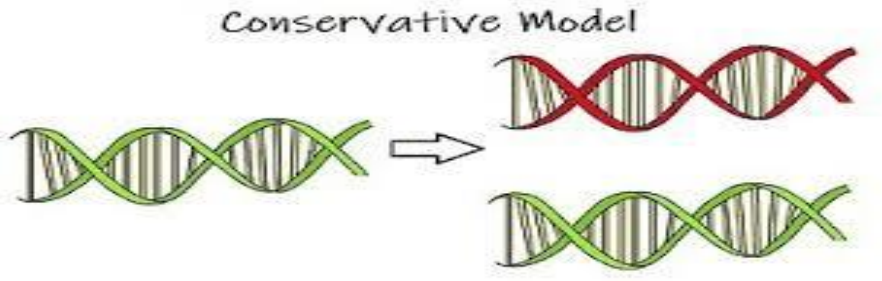
Semi-Conservative Model :

According to this paradigm, the two strands of DNA unwind from one another, each acting as a template for developing a brand-new, complementary strand of DNA. Due to this, two DNA molecules are created, one of which has the original strand and the other containing a new one.



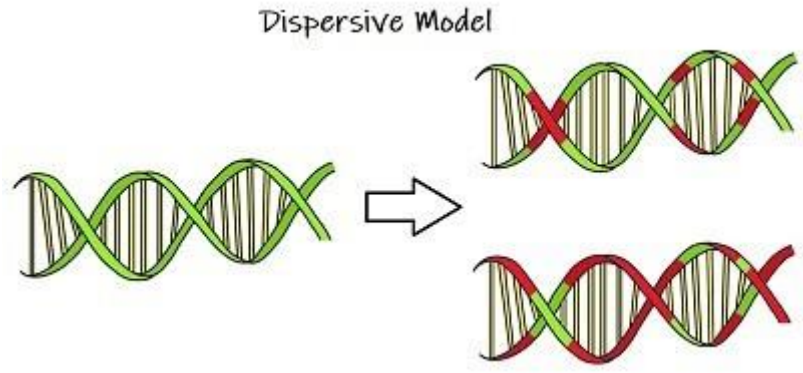
Conservative Model:

In this case, DNA replication creates two new molecules, one containing two new DNA strands and is not identical to the original DNA molecule. In contrast, the original DNA molecule retains its two original strands with exactly the same sequences as the original molecule.



Dispersive Model:

Dispersive model: According to this theory, each strand is composed of a patchwork of genetic material that was original and recently synthesised.



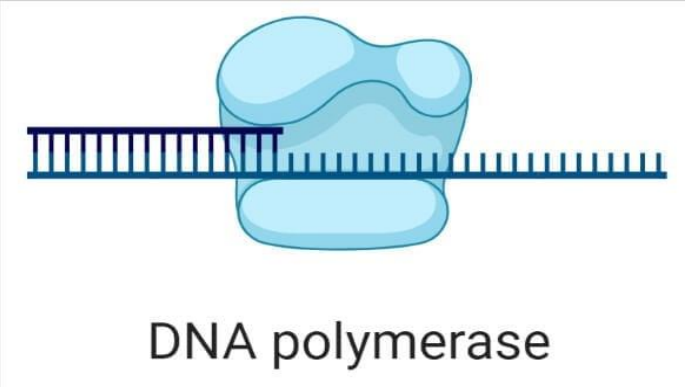
ENZYMES INVOLVED IN DNA REPLICATION

Enzymes play a major role in DNA replication because they catalyze several important stages of the entire process. They are 7 enzymes and proteins used in DNA Replication

Nucleases

- A nuclease is an enzyme that can cleave the phosphodiester bonds present in between the nucleotides.
- On the basis where they cleave, they are characterized as Exo and endonucleases.
- Exonucleases cleave nucleotides from their respective ends. Corresponding to this fact, these exonucleases show activity from both directions 5′ to 3′ and 3′ to 5′.
- Endonucleases act on the region in the middle of the targeted nucleotide. They are also endonucleases that are selective to which molecule they cleave and are sub-divided as DNase for DNA for cleaving and RNase for RNA cleaving. Additionally, recently discovered nucleases are also being used for gene editing such as Cas9 in the CRISPR genome editing technique.
- Restrictive endonuclease or restriction enzymes are the ones that cleave DNA into fragments at or near the specific recognition sites within the molecule known as restriction sites.
- To cleave the DNA, restriction endonuclease makes two incisions, once through each sugar-phosphate backbone of the DNA double helix. These endonucleases recognize a specific sequence of nucleotides and produce a double-stranded cut in the DNA.
- This specific sequence is usually 4 – 8 bases and is present in the recognition site.

DNA polymerase



- DNA polymerases are the enzyme that is responsible for adding new nucleotides and synthesizing a new strand of DNA by taking the old fragmented strand as a template.
- DNA Polymerases also possess exonuclease activity, that cuts incorrectly added nucleotides, and allows the DNA replication to happen without errors.
- DNA Polymerase is of many types and functions based on the cell they are found in.
- In prokaryotic cells, there are three DNA polymerases:
- DNA Polymerase I, DNA Polymerase II and DNA Polymerase III.
- DNA polymerase I is a repair polymerase with 5′ to 3′ and 3′ to 5′ exonuclease activity. It is involved in the processing of Okazaki fragments during lagging strand synthesis.
- DNA polymerase II has 3′ to 5′ exonuclease activity and participated in DNA repair with 5′ to 3′ polymerase activity.
- DNA polymerase III is the primary enzyme involved in the DNA replication of *E.coli*. It has 3′ to 5′ exonuclease activity and 5′ to 3′ polymerase activity.
- In eukaryotic cells, there are five DNA polymerases: DNA Polymerase α, β, γ, δ and ε
- DNA polymerase α is a repair polymerase, with 3′ to 5′ exonucleases activities and 5′ to 3′ polymerase activities.
- DNA Polymerase β is a repair polymerase.
- DNA Polymerase γ shows polymerase activity 5′ to 3′ and exonucleases activity 3′ to 5′, it is involved in Mitochondrial DNA replication
- DNA Polymerase δ shows 3′ to 5′ exonuclease activity and 5′ to 3′ polymerase activity. This enzyme is involved in lagging strand synthesis.
- DNA Polymerase ε shows 3′ to 5′ and 5′ to 3′ exonucleases activities. This enzyme not only repairs but also synthesizes the leading strand efficiently in a 5′ to 3′ direction. It is the prime enzyme involved in DNA replication.

DNA ligase

- DNA ligase is a specific type of enzyme that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- This enzyme joins the 3′ hydroxyl group of one nucleotide with the 5′ phosphate end of another nucleotide at an expense of ATP.

DNA helicase

- DNA helicase is a motor protein that moves directionally along a nucleic acid phosphodiester backbone, separating two nucleotides of DNA molecule.
- They separate double-stranded DNA molecules into single strands allowing each strand to be copied.
- During DNA replication, this DNA helicase unwinds DNA at the origin, a site where the replication is to be initiated.
- DNA helicase continues to unwind the double helix of DNA and thus forms a structure called replication fork, named after the forked appearance of two strands of DNA when unzipped apart.
- It is an energy-driven process as it involves the breaking of Hydrogen bonds between annealed nucleotide bases.

DNA primase

- Primase is an enzyme that is capable to synthesize short stretches of RNA sequences known as a primer.
- Primers are an integral part of DNA replication. These primers serve as an initiating site for the addition of nucleotides by DNA polymerase.
- DNA polymerase can only add nucleotide at pre-existing 3′ Hydroxyl group which is thus provided by the primers.
- As we can see that primers are short stretches of RNA, but replication is of DNA, so therefore after elongation of the chains of nucleotides, these primers are replaced by DNA.

DNA topoisomerase

- DNA topoisomerase is a class of enzymes that release helical tension during transcription and replication by creating transient nicks within the phosphate backbone on one or both strands of the DNA.

- This tension is aroused when the DNA molecule unwinds due to helicase activity and forms a replication fork. The progress of the replication fork generates supercoils, making it hard for other machinery involved to access the DNA molecule.
- Class I DNA topoisomerase makes a single-stranded break to relax the helix and progress the process.
- Class II DNA topoisomerase break both the strands of DNA helix, this class of topoisomerases is also very important during the cell cycle for the condensation of chromosomes.

Single strand binding proteins

- The single-strand binding (SSB) proteins are DNA binding proteins, that binds to single-stranded DNA to facilitate DNA replication.
- SSB proteins prevent the hardening of strands during DNA replication. It also protects strands from nuclease degradation and prevents the rewinding of DNA.
- These proteins destabilize helical duplexes so that DNA polymerase can hold onto the DNA during DNA replication, recombination, and repair.
- It also removes unwanted secondary structures on strands for easy access of the strands to the machinery involved in DNA replication.
- Thus, SSB proteins stabilize the single-stranded DNA structure that is important for genomic progression.

ROLLING CIRCLE REPLICATION

It describes a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of plasmids.

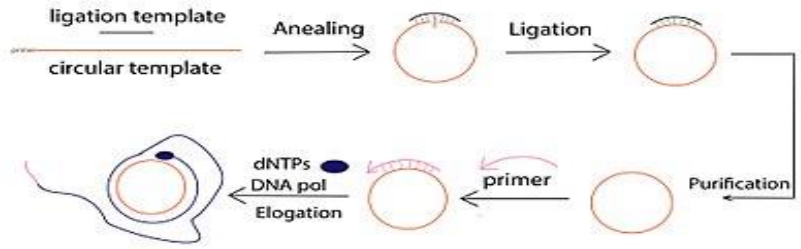
Rolling circle DNA replication is initiated by an initiator protein encoded by the plasmid or bacteriophage DNA, which nicks one strand of the double-stranded, circular DNA molecule at a site called the double-strand origin, or DSO. The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a primer for DNA synthesis by DNA polymerase III. Using the unnicked strand as a template, replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA. Displacement of the nicked strand is carried out by a host-encoded helicase called PcrA (the abbreviation standing for plasmid copy reduced) in the presence of the plasmid replication initiation protein.

Continued DNA synthesis can produce multiple single-stranded linear copies of the original DNA in a continuous head-to-tail series called a concatemer. These linear copies can be converted to double-stranded circular molecules through the following process:

First, the initiator protein makes another nick in the DNA to terminate synthesis of the first (leading) strand. RNA polymerase and DNA polymerase III then replicate the single-stranded origin (SSO) DNA to make another double-stranded circle. DNA polymerase I removes the primer, replacing it with DNA, and DNA ligase joins the ends to make another molecule of double-stranded circular DNA.

As a summary, a typical DNA rolling circle replication has five steps:

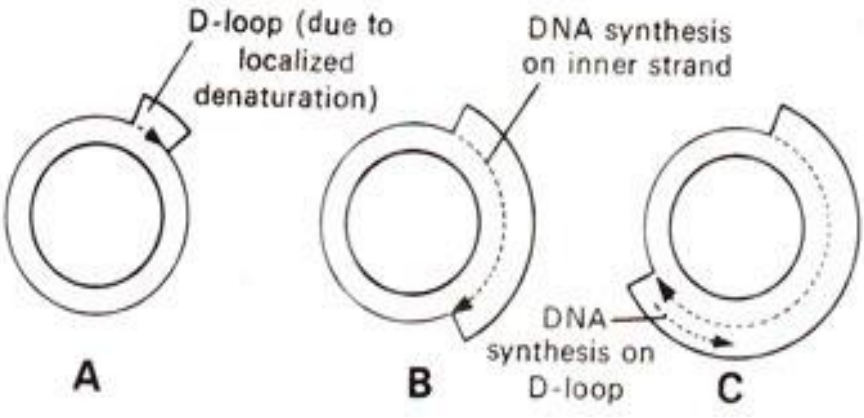
1. Circular dsDNA will be "nicked".
2. The 3' end is elongated using "unnicked" DNA as leading strand (template); 5' end is displaced.
3. Displaced DNA is a lagging strand and is made double stranded via a series of Okazaki fragments.
4. Replication of both "unnicked" and displaced ssDNA.
5. Displaced DNA circularizes.



D – LOOP REPLICATION

D- loop is a DNA structure where the two strands of a double-stranded DNA molecule are separated for a stretched and held apart by third strand of DNA.

- In some of the strands which are designated as crossovers, a different sequence of events occurs.
- The first detectable step of these series of events is the sequence is the stabilization, by unknown means,of interaction between the invading single-strand and its homolog—resulting in a structure called a single-end invasion.
- The extension of the single strand then leads to expansion of the D-loop, interacting with the second single-stranded end generated at the original double-strand break.
- Further DNA synthesis and ligation fill in the gaps, and the final result is a DNA structure called a double Holliday junction.
- Specific pair of DNA cleavage reactions, followed by DNA repair, transform this structure into a crossover.



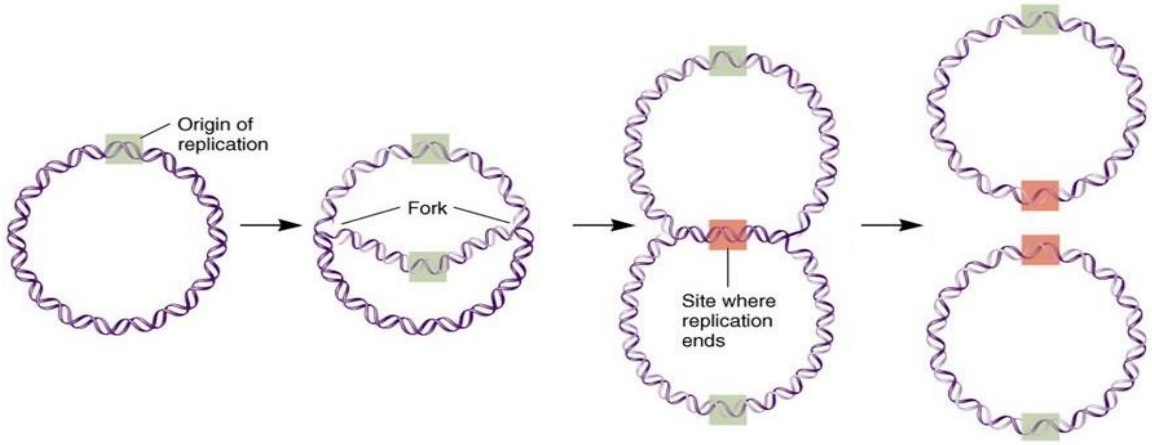
THETA MODEL DNA REPLICATION

The theta mode of replication is a type of replication that is seen in circular DNA molecules. A circular chromosome is a form of circular DNA found in bacteria and archaea that has no free ends, unlike the linear DNA strands seen in most eukaryotes.

There are three types of replication modes in circular DNA: rolling-circle, strand displacement, and theta. Let us discuss the theta mode of replication briefly.

The theta mode of replication is similar to the linear mode of chromosome replication, where leading strands are replicated continuously, and lagging strands are replicated discontinuously. No DNA breaks are required in this mode of replication.

The theta mode of replication is commonly found in bacteria, such as *Escherichia coli* and Bacillus subtilis, as well as in chloroplast and mitochondria. It is referred to as the theta mode of replication because of an intermediate form that resembles the Greek letter θ.



UNIT - 2

DNA DAMAGE

DNA is the basic unit of inheritance that maintains the integrity and function of living organisms. However, it is constantly exposed to damaging agents which can cause DNA damage. Additionally, errors can occur during DNA replication and repair processes, leading to harmful mutations

To overcome the harmful effects of DNA damage, cells have various systems such as DNA repair mechanisms, damage tolerance pathways, cell cycle checkpoints, and cell death pathways. These systems work together to repair or tolerate DNA damage, ensuring the overall survival and functionality of cells.

Understanding the mechanisms of DNA damage and the corresponding repair pathways is essential to understand the impact of DNA damage on cellular functions and the development of diseases.

TYPES OF DNA DAMAGE AND MECHANISMS

There are various types of DNA damage that can happen due to normal cellular processes or exposure to damaging agents in the environment.

Several types of DNA damage are described as follows:

1. DNA Strand Breaks

DNA strand breaks occur when one or both strands of DNA are interrupted. There are two types: single-strand breaks (SSBs) where one strand is cut, and double-strand breaks (DSBs) where both strands are cut. These breaks can be caused by ionizing radiation like X-rays and gamma rays, as well as certain chemicals.

2. Oxidative Damage

Oxidative damage can occur due to the action of reactive oxygen species (ROS) which leads to the formation of lesions. The highly reactive ROS, such as hydroxyl radicals (•OH), can cause oxidative damage to DNA bases.

3. Alkylation of Bases

Alkylating agents, both endogenous and exogenous, can modify DNA bases by introducing alkyl groups. These modifications can be cytotoxic, mutagenic, or have neutral effects on the cell.

4. Base Loss

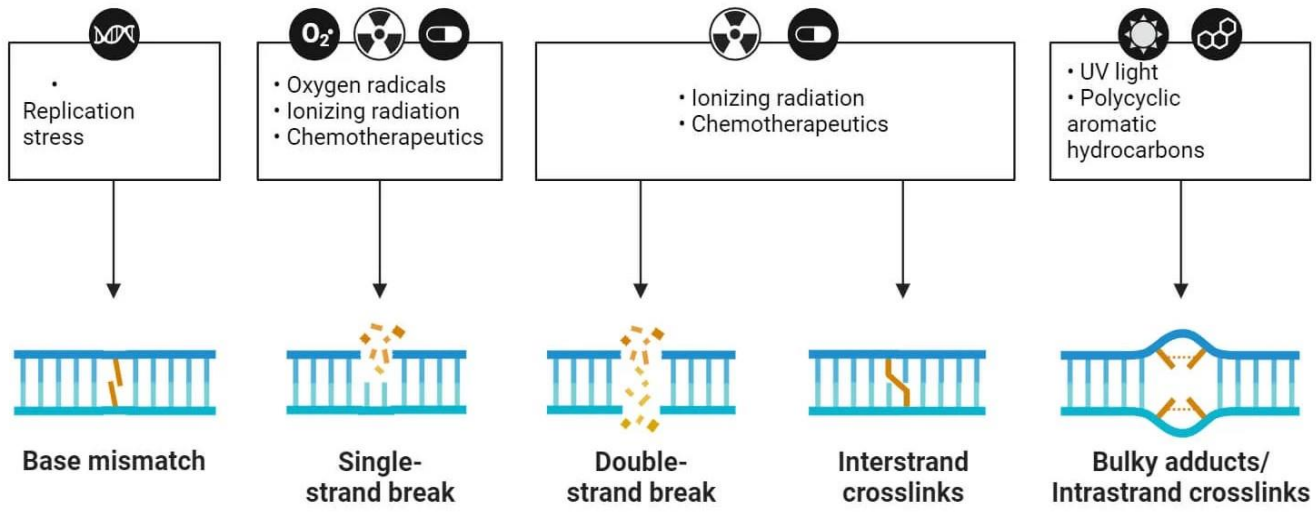
Base loss occurs when the nitrogenous bases in DNA are removed, leaving behind apurinic/apyrimidinic (AP) sites or abasic sites. AP sites are chemically unstable and can lead to DNA strand breaks or mutagenic events if left unrepaired.

5. Bulky Adduct Formation

Bulky adducts are formed when certain chemicals, such as polycyclic aromatic hydrocarbons (PAHs), covalently bind to DNA bases. These adducts create bulky modifications that stick out from the DNA and disrupt its structure. They can interfere with DNA replication, transcription, and repair processes, potentially leading to mutations.

6. DNA Crosslinking

DNA crosslinking occurs when two nucleotides in DNA become covalently linked together. Crosslinks can form within the same DNA strand (intrastrand crosslinks) or between opposite DNA strands (interstrand crosslinks). DNA crosslinks prevent the separation of DNA strands during replication or transcription, leading to the disruption of important cellular processes.



SOURCES/ AGENTS OF DNA DAMAGE

DNA damage can also be classified into two types based on its origin or sources: endogenous and exogenous. The major sources of endogenous and exogenous DNA damage are briefly explained below:

1. Endogenous DNA Damage

Endogenous DNA damage originates from internal reactions involving chemically active DNA within cells.

- **Replication errors** are one source of endogenous DNA damage that occurs during DNA replication when incorrect nucleotides are inserted opposite the template bases. During replication, some DNA polymerases with lower fidelity can be involved, leading to potential errors.

- **Topoisomerase enzymes** are another source of endogenous DNA damage.Topoisomerases remove the supercoiling of DNA during replication and transcription. However, misalignment of the DNA ends can stabilize the topoisomerase-DNA cleavage complex and result in the formation of DNA lesions.
- **Reactive oxygen species (ROS)** are produced during cellular processes and can cause oxidative damage to DNA. While ROS plays an important role in normal cellular functions, excessive levels can lead to various DNA lesions and modifications. Excessive ROS has been associated with the development of several human diseases like cancer, Alzheimer's disease, and diabetes.
- **Alkylating agents** are reactive compounds that can add methyl or ethyl groups to DNA bases, leading to chemical modifications. Spontaneous methylation events can generate different methylated bases. Some methylated bases are mutagenic and can lead to specific types of mutations.

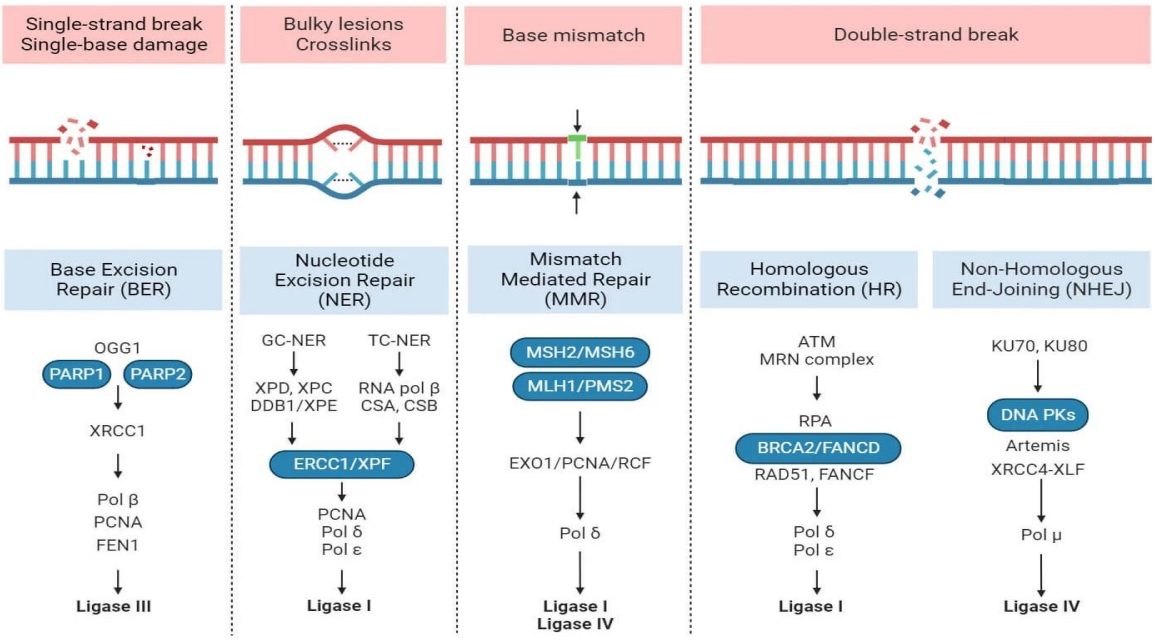
2. Exogenous DNA Damage

Exogenous DNA damage is caused by external factors, such as environmental agents, physical forces, or chemicals.

- **Ionizing radiation (IR)** directly damages DNA or indirectly affects it through the generation of highly reactive hydroxyl radicals (•OH) from water molecules. IR can cause different types of damage to the DNA such as base lesions, and single-strand and double-strand breaks.
- **Ultraviolet (UV) radiation** is another agent of DNA damage. It is the leading cause of skin cancers. UV light can form pyrimidine dimers where two pyrimidines on the same DNA strand are joined together. This alteration in DNA structure can block transcription and replication processes.
- **Exogenous alkylating agents**, found in sources like tobacco smoke and industrial activities, react with DNA and can cause mutagenic and carcinogenic changes. They primarily target the nitrogenous bases in DNA. Examples of alkylating agents include sulfur and nitrogen mustards.
- **Aromatic amines**, found in cigarette smoke, fuel, coal, dyes, and pesticides, are also exogenous sources of DNA damage. These agents can create long-lasting lesions in the DNA structure that lead to the substitution of DNA bases and frameshift mutations.
- **Polycyclic aromatic hydrocarbons (PAHs)** are known carcinogens found in sources like tobacco smoke, automobile exhaust, and other environmental pollutants. PAHs require activation by the liver's P-450 system to produce reactive substances that can potentially damage DNA.

DNA REPAIR MECHANISMS

DNA damage is a common event that can interfere with important cellular processes and lead to genomic defects and an increased risk of cancer. To ensure the integrity of their genomes, cells have evolved to develop mechanisms for DNA repair. These mechanisms help cells to cope with DNA damage.

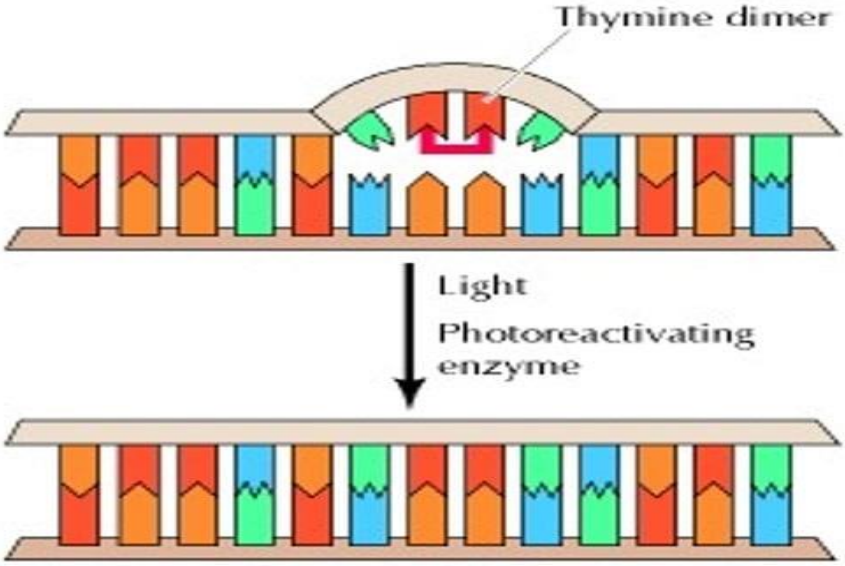


TYPES OF DNA REPAIR MECHANSIMS

Various pathways exist for DNA repair. These include direct reversal, excision repair, mismatch repair, and repair of DNA breaks.

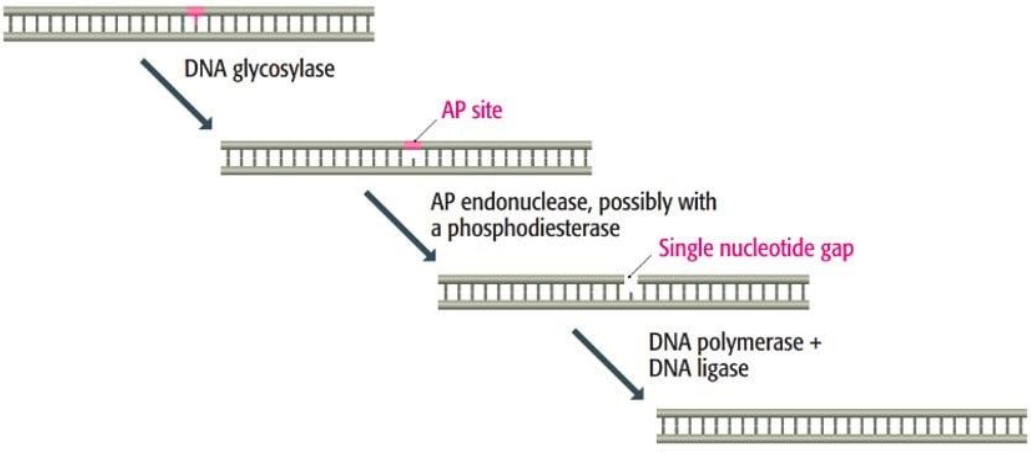
1. Direct reversal repair

- Direct reversal repair is a DNA repair mechanism that directly fixes specific types of DNA damage without the need for excision or replacement.
- Two examples of DNA damage that can be reversed are UV-induced lesions and alkylated bases.
- UV-induced lesions, caused by UV light, can be reversed through a process called photoreactivation, which uses visible light energy to break the damaged DNA structure, restoring the original pyrimidine bases.
- Alkylated bases can be reversed by enzymes such as O^6-alkylguanine-DNA alkyltransferase (AGT) and AlkB-related dioxygenases, which removes or modifies the alkyl group, respectively.



2. Base excision repair

- Base excision repair (BER) is a DNA repair mechanism that removes and replaces damaged bases.
- It involves the action of various DNA glycosylases such as 8-oxoguanine DNA glycosylase (OGG1). These enzymes recognize and remove damaged bases.
- BER includes both short patch repair, where an abasic site is processed and filled by specific enzymes, and long patch repair, where gaps are tailored and DNA synthesis occurs followed by ligation.
- One example of BER is the repair of uracil-containing DNA. In this process, a DNA glycosylase recognizes and removes the uracil base, creating a gap in the DNA called AP site. The gap is then cleaved by an enzyme called AP endonuclease. After that, the remaining sugar is removed, and the gap is filled using DNA polymerase and sealed with ligase.

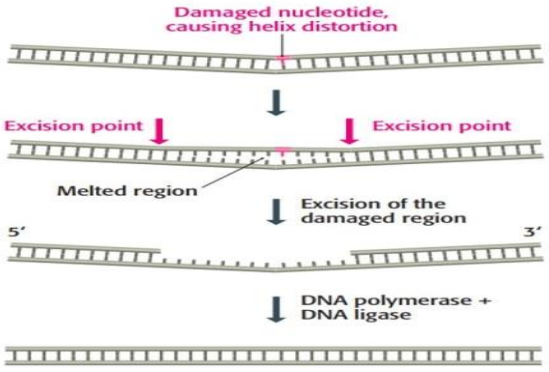


3. Nucleotide excision repair

- Nucleotide excision repair (NER) deals with bulky adducts and cross-linking lesions caused by UV radiation or chemical exposure.
- NER removes a fragment of nucleotides containing the damaged lesion and synthesizes a new DNA strand using the undamaged strand as a template.

NER consists of two pathways:

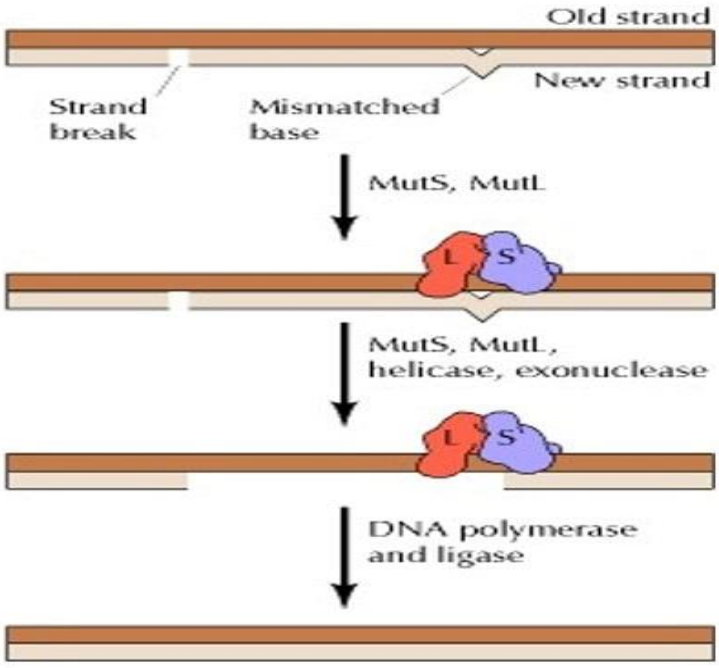
- Global Genome NER (GG-NER) repairs bulky damages throughout the entire genome, including regions that are not actively transcribed.
- Transcription-Coupled NER (TC-NER repairs damage that occurs on the transcribed DNA strand.
- Mutations in NER pathway genes can lead to disorders such as xeroderma pigmentosum (XP) and certain other neurodegenerative conditions.



4. Mismatch repair

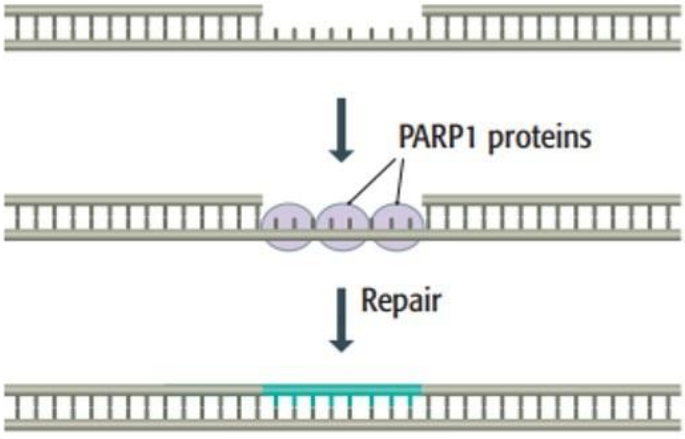
- Mismatch repair (MMR) pathway repairs base mismatches and insertion-deletion loops that occur during replication. Most of these errors are fixed by the proofreading activity of DNA polymerase during replication, but some may be missed and need to be corrected later.
- The MMR pathway involves three steps: recognition of mismatches, degradation of the error-containing strand, and synthesis of the correct DNA sequence.
- First, protein complexes such as MSH2-MSH6 in the MutS protein locate the mismatch errors and forms a complex with MutL which helps in further repair. MutS and MutL are important protein complexes in eukaryotes. In E. coli, another protein MutH also has an important role in mismatch repair.
- Next, exonuclease 1 (Exo1) degrades the error-containing strand while replication protein A (RPA) prevents further DNA degradation by binding to the exposed DNA.
-

- Then, DNA polymerase δ synthesizes the correct sequence. Finally, DNA ligase then seals any remaining nicks in the repaired DNA.
- Mutations in MMR genes can lead to Lynch syndrome, a hereditary condition associated with an increased risk of colon, ovarian, and other cancers.



5. Single-strand break repair (SSBR)

- Single-stranded breaks (SSBs) in DNA can occur due to oxidative damage, abasic sites, or errors in the activity of the DNA topoisomerase enzyme.
- These breaks can disrupt DNA replication, halt transcription, and activate cellular processes that can lead to cell death.
- To protect the exposed single strand from breaking, PARP1 proteins coat the single strand and act as a shield.
- SSBR can be accomplished through various pathways already explained above, including base excision repair, nucleotide excision repair, and mismatch repair.



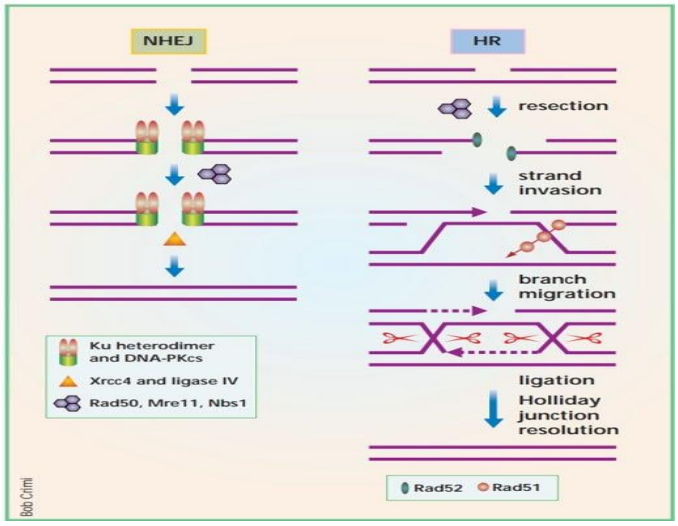
6. Double-strand break repair

Double-strand breaks (DSBs) in DNA can be repaired through two pathways: homologous recombination (HR) and non-homologous end joining (NHEJ).

Homologous recombination (HR)

- HR is a precise repair pathway that requires a matching DNA sequence as a template.
- It primarily uses the sister chromatid, a copy of the damaged DNA, for repair.
- HR is most active during the S, G2, and M phases of the cell cycle when sister chromatids are present.

- The HR process involves creating single-stranded DNA (ssDNA) by degrading one strand of the DNA break and coating it with proteins like RPA. Rad51 replaces RPA and pairs the ssDNA with a homologous DNA template for repair.



Non-homologous end joining (NHEJ)

- NHEJ is a simple and widely used mechanism that directly seals the broken ends of DNA without the need for a homologous DNA template.
- It can occur throughout the cell cycle.
- Proteins like Ku70/Ku80, DNA-PKcs, and LIG4/XRCC4 are involved in NHEJ. Ku70/Ku80 protects the DNA ends and prevents recombination, while DNA-PKcs and LIG4/XRCC4 help with end joining.
- The NHEJ pathway is faster but can be more error-prone compared to HR

INTRODUCTION TO RECOMBINATION

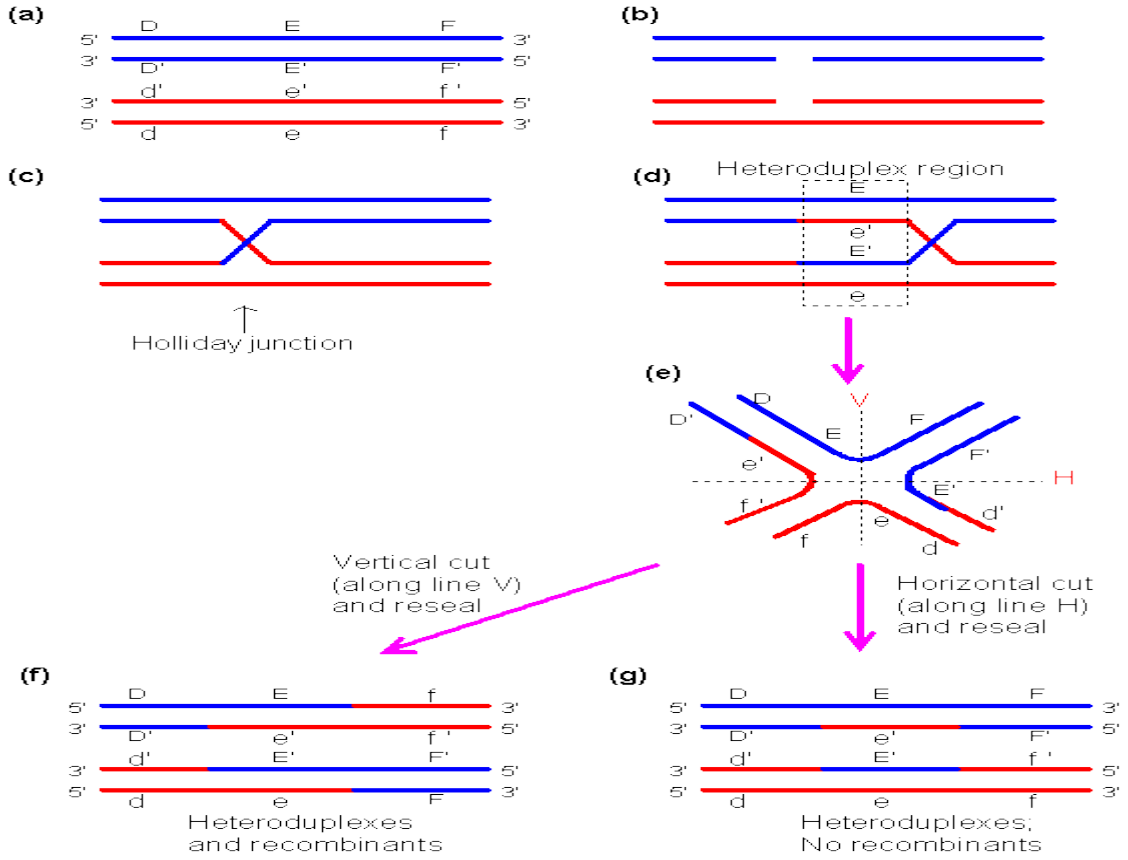
In general recombination, the exchange of genetic material (DNA) takes place between a pair of homologous DNA sequences. These sequences are located on two copies of the same chromosome. However, in some cases, the DNA molecules that share the same nucleotide sequence can also recombine. The process of general recombination reaction is essential for every dividing cell because mistakes occurring occur during DNA replication that interrupts the replication fork must be repaired for general recombination mechanisms. Recombination process occurs naturally during meiosis and can also be performed in the laboratory. Recombination leads to an increase in the genetic diversity in sexually reproducing organisms.

Holliday Model

In 1964, Robin Holliday proposed a model that accounted for heteroduplex formation and gene conversion during recombination. Although it has been supplanted by the double-strand break model (at least for recombination in yeast and higher organisms), it is a useful place to start. It illustrates the critical steps of pairing of homologous duplexes, formation of a heteroduplex, formation of the recombination joint, branch migration and resolution.

The steps in the Holliday Model are illustrated in Figure 8.6.

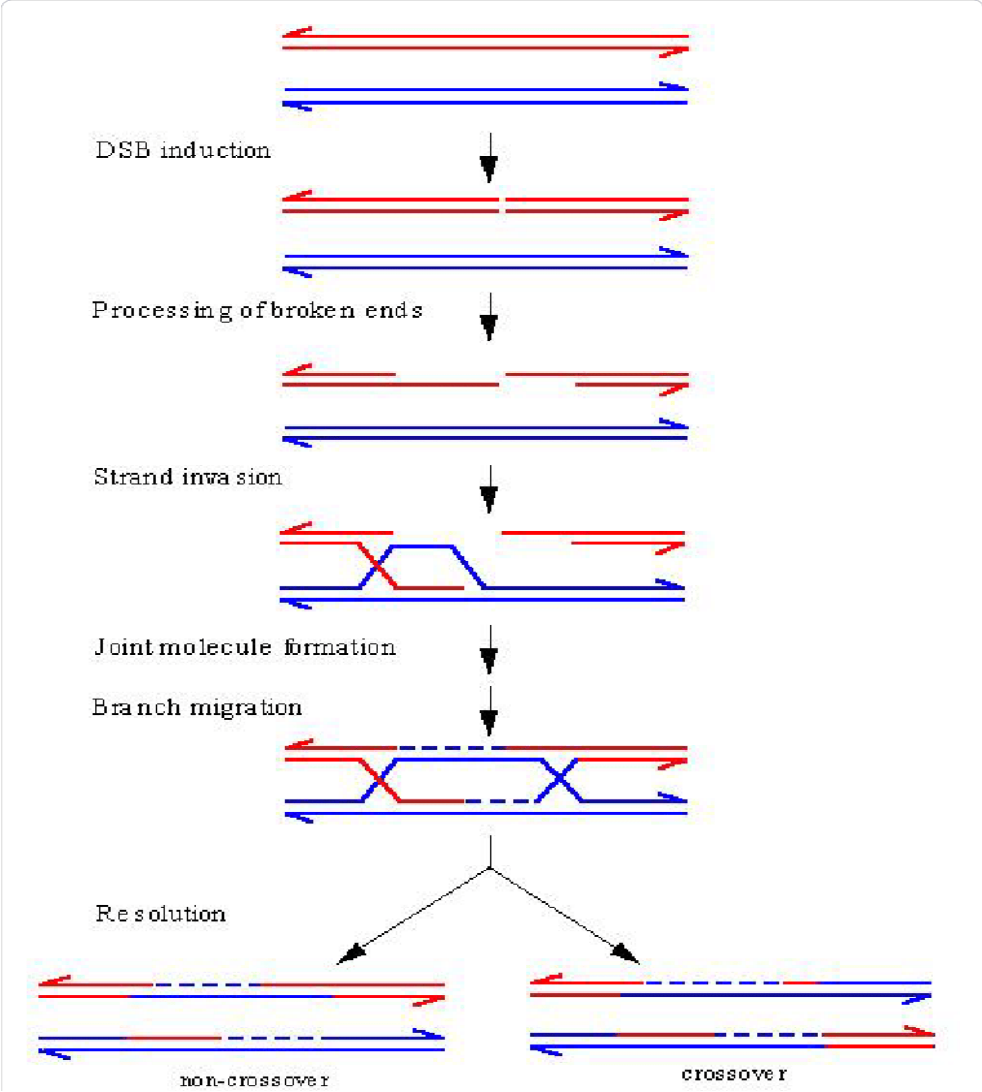
1. Two homologous chromosomes, each composed of duplex DNA, are **paired** with similar sequences adjacent to each other.
2. An **endonuclease nicks** at corresponding regions of homologous strands of the paired duplexes. This is shown for the strands with the arrow to the right in the figure.
3. The nicked ends dissociate from their complementary strands and each **single strand invades the other duplex**. This occurs in a reciprocal manner to produce a **heteroduplex region**derived from one strand from each parental duplex.
4. DNA ligase **seals the nicks**. The result is a stable **joint molecule**, in which one strand of each parental duplex crosses over into the other duplex.This X-shaped joint is called a **Holliday intermediate** or **Chi structure**.
5. Branch migration then expands the region of heteroduplex. The stable joint can move along the paired duplexes, feeding in more of each invading strand and extending the region of heteroduplex.
6. The recombination intermediate is then **resolved** by nicking a strand in each duplex and ligation.



DOUBLE STRANDED BREAK REPAIR

- The internal environment of a cell is not nearly as calm and predictable as we have been led to believe, in fact, chemical, mechanical and energetic stressors are constantly bombarding the cell.
- These factors also impact DNA within cells often causing single-strand and Double-Stranded Breaks (also referred to as DSBs) in the DNA molecule! A DSB occurs when the phosphorous-sugar backbone of the DNA molecule is cleaved between 2 neighboring nucleotides .

- It is important to note sometimes DSBs are intentionally initiated by cells for events such as crossing over, but this occurs only in meiotic cells which perform crossing-over to produce gametes.
- However, non-meiotic cells regularly experience DSBs, despite their DNA being safely tucked away in the nucleus, in fact, biologists estimate that in every active cell there are at least ten double-stranded DNA breaks per day and single-strand breaks happen at a "much" higher rate!
- These DSBs are extremely cytotoxic and potentially very damaging to the cell, and of all the types of damage that can occur in cells DSBs are considered the most dangerous!
- DSBs are extremely cytotoxic, as they can block DNA replication, cause the loss of a chromosome as well as lead to cell death. Given that cells rely on DNA for their very existence
- It is obvious that all cells must possess a high-fidelity mechanism to repair such damage and this is called **double strand DNA break repair.**



TRANSPOSABLE ELEMENTS

Transposable elements (TE) or transposons can be defined as small, mobile DNA sequences that move around chromosomes with no regard for homology, and insertion of these elements may produce deletions, inversions, chromosomal fusions, and even more complicated rearrangements.

- Transposons are mobile genetic elements that often carry an antimicrobial resistance gene.

- These elements can insert randomly, move from plasmids to the chromosome, and vice versa, and can be moved from one bacterium to another by conjugation, transformation, or transduction.
- Transposable elements make up a significant fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell.
- Transposable elements were discovered by Barbara McClintock (1965) through an analysis of genetic instability in maize (corn).

CHARATERISTICS OF TRANSPOSABLE ELEMENTS

Some salient features of transposable elements are:

1. These are the DNA sequences that code for enzymes which result in self-duplication and insertion into a new DNA site.
2. Transposons are involved in transposition events which include both recombination and replication, which usually generates two copies of the original transposable elements. One of the copies remains at the parent site, whereas the other one reaches the target site on the host chromosome.
3. The integrity of the target genes of these elements is invariably disrupted by the presence of those elements.
4. Because transposons carry the genes for initiation of RNA synthesis, some previously dormant genes might be activated.
5. A transposable element doesn't have a site for the origin of replication. As a result, it cannot replicate without the host chromosome as plasmids or phages.
6. There is no homology between the transposon and its target site for insertion. These elements can insert at almost any position in the host chromosome or a plasmid. Some transposons might seem likely to enter at some specific positions (hot spots), they barely insert at base-specific target sites.

UNIT-3

Transcription

"Transcription is the first step of gene expression that involves the formation of RNA molecule from DNA.

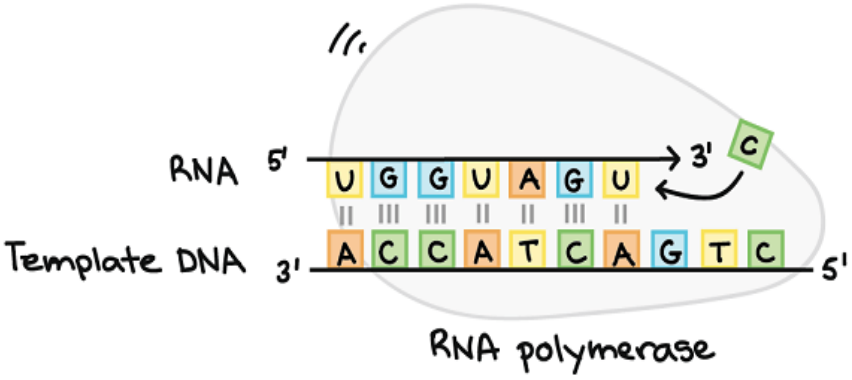
It is one of the first processes in gene expression. The genetic information flows from DNA to protein and this flow of information takes place in a sequential process of transcription and translation. Only one strand of DNA is copied during the process of transcription known as the template strand and the RNA synthesized is called the mRNA.

The main motive of transcription is RNA synthesis from the DNA sequence. The RNA transcript carries the information used to encode a protein.

RNA polymerase

The RNA polymerase is the main enzyme involved in transcription. It uses single-strand DNA to synthesize a complementary RNA strand. The DNA-dependent **RNA polymerase** binds to the promoter and catalyses the polymerization in the 5' to 3' direction on the template strand. Once it reaches the terminator sequence, the process terminates and the newly synthesized RNA strand is released.

Transcription Unit is a stretch of a DNA transcribed into an RNA molecule. Its function is to encode at least one gene. Suppose if gene encodes protein than mRNA is produced by transcription. A protein encoded by the DNA transcription unit may comprise a coding sequence. Compared to DNA replication, transcription has a lower copying fidelity.



DNA Transcription Process

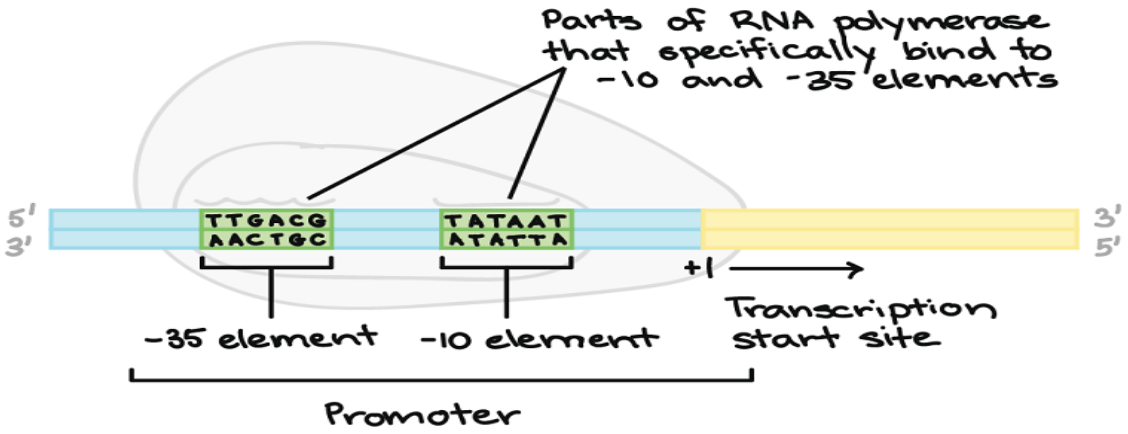
- In prokaryotic cells, the entire mechanism of transcription is summarized in three stages: Initiation, Elongation, and Termination.
- At the end of the termination in prokaryotes, the mRNA formed is ready for translation.
- Unlike in eukaryotes, after termination, an immature mRNA is formed, and therefore, more processes are needed to form a mature mRNA which is then translated into proteins.
- Generally, the transcription process transcribes DNA into mRNA, the type of RNA that carries the information that is needed in the synthesis of proteins.
- In eukaryotes, there are two broad steps that take place in transcription;
    - Pre-messenger RNA formation using an RNA polymerase enzyme
    - Editing of pre-messenger RNA by splicing
- Pre-messenger RNA formation involves the initiation, elongation, and termination phases which end by forming mRNA.
- The mRNA then undergoes different stages of splicing to form mature mRNA.

Formation of pre-messenger RNA

- When transcription starts, DNA must unwind, aided by RNA polymerase, which catalyzes the process.
- In transcription only one of the DNA strands is transcribed, the strand that has the initiator sequence. this strand is known as the sense strand, while the complementary strand is known as the antisense strand.
- mRNA that is transcribed is normally a copy of the sense strand, however, it is the antisense strand that is transcribed.
- The ribonucleoside triphosphate (NTPs) aligns along the antisense DNA strand by base pairing, then the RNA polymerase joins the ribonucleotides together forming a pre-messenger RNA molecule, complementary to a region on the antisense strand.
- Transcription is completed when the RNA polymerase enzyme finds a triplet of bases read as a stop signal. AT this stage, the DNA molecule rewinds to reform the double helix.
- The formation of the messenger RNA (mRNA) is done in three stages: Initiation, elongation, and termination.

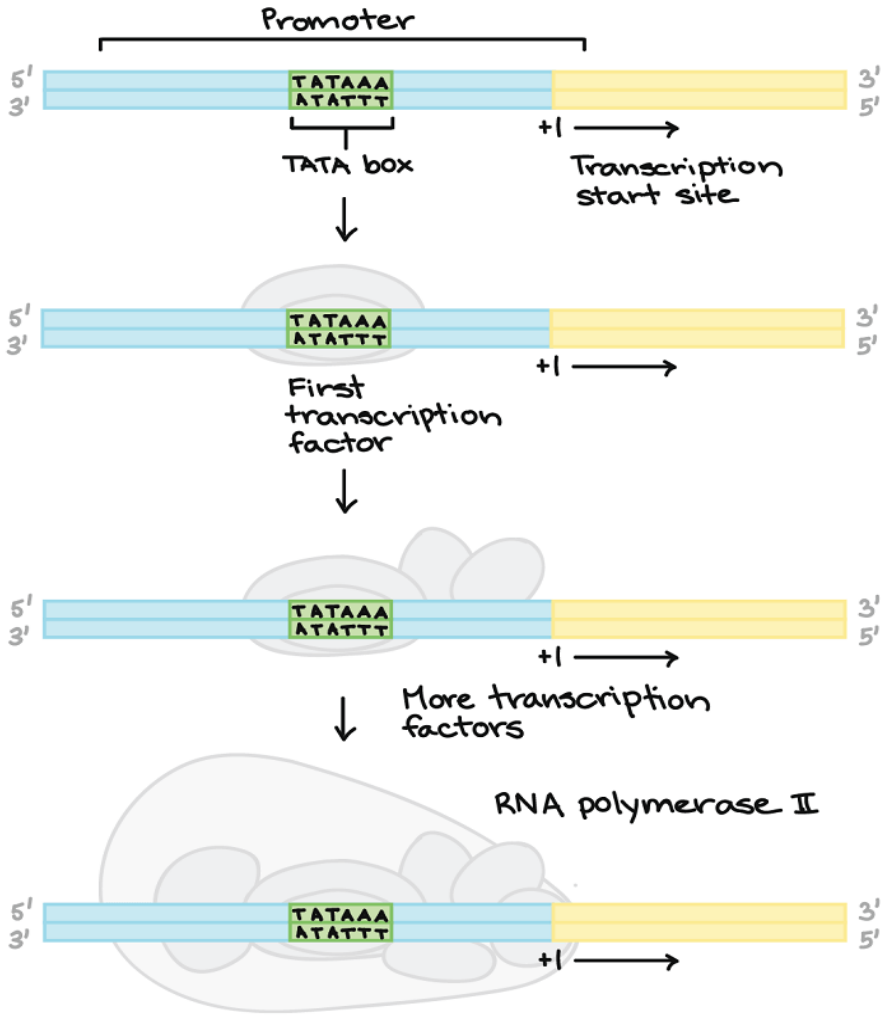
Initiation

Promoter and initiation in prokaryotes



- The initiation of transcription is signaled at a region known as a promoter.
- The promoter is the site for RNA polymerase binding, such that the promoter guides the polymerase where it should sit on the DNA in order to initiate transcription.
- RNA polymerase is the enzyme that catalyzes the mechanism of transcription.
- The RNA polymerase enzyme has a sigma (σ) factor, which is the dissociative unit, that allows the enzyme to recognize the promoter sequence (starting point of transcription), which is spaced between -35 and -10 regions.
- The promoter sequence is recognized by RNA polymerase's holoenzyme subunit, by attaching to and moving along the DNA template molecule. This forms a **closed promoter complex.**
- A single DNA molecule may have multiple promoter sequences or closed promoter complexes.
- The promoter which is bound with transcription factors along with the RNA polymerase forms a complex.
- The transcription factors are regulatory proteins that control transcription rate.
- When the RNA polymerase is bound to the promoter sequence, it denaturalizes the DNA duplex locally, forming open promoter complex which becomes the unwound part of the double-stranded DNA, exposing the bases on each of the two DNA strands.

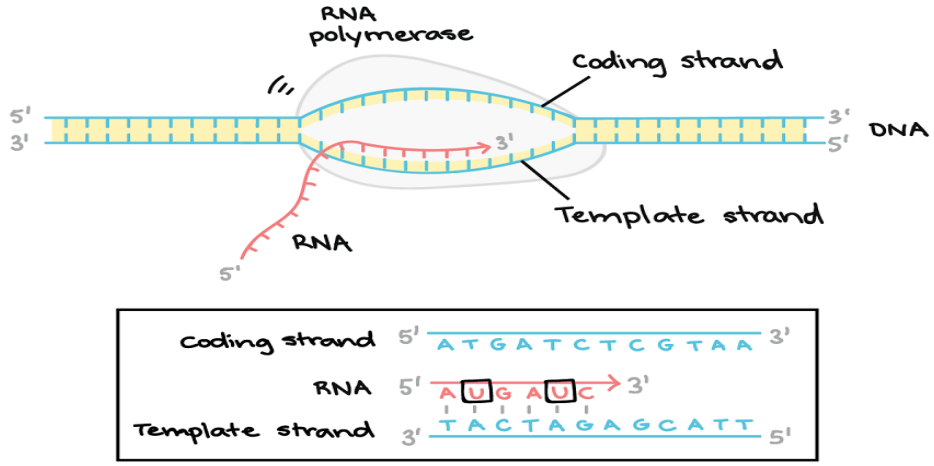
Promoter and initiation in Eukaryotes



- In eukaryotes, the RNA polymerase does not directly attach to the promoter sequence like in prokaryotes.
- A helper promoter known as a basal (general) transcription factor binds to the promoter first, which helps the RNA polymerase attach to the DNA template.
-

- Eukaryotes have a promoter sequence called a **TATA box,** which is recognized by the transcription factors, which eventually allow the binding of the RNA polymerase.
- The TATA box has lots of As and Ts making it easy to pull the strands of DNA apart.

Elongation



- After initiating transcription, the sigma (σ) factor dissociates from the RNA polymerase.
- The template strand is read in the 3′ to 5′ direction, which means that RNA synthesis takes place in the 5′ to 3′ direction, with the nucleoside triphosphate (NTPs) acting as substrates for the enzyme.
- The other strand from the DNA template is known as the coding strand, because the base sequence of the new mRNA is identical to it, except for the replacement of thiamine with uracil base.
- The RNA polymerase catalyzes the formation of a phosphodiester bond between the adjacent ribonucleotides.
- The energy used by the RNA polymerase is derived from splitting the high-energy triphosphate into monophosphate, releasing the inorganic diphosphates (PPi).
- A transcription bubble is formed and it must be maintained sine transcription takes place on the double-stranded DNA template. The bubble moves along the DNA duplex during elongation.
- Stalling or pausing are common, which are later essential for transcription termination.

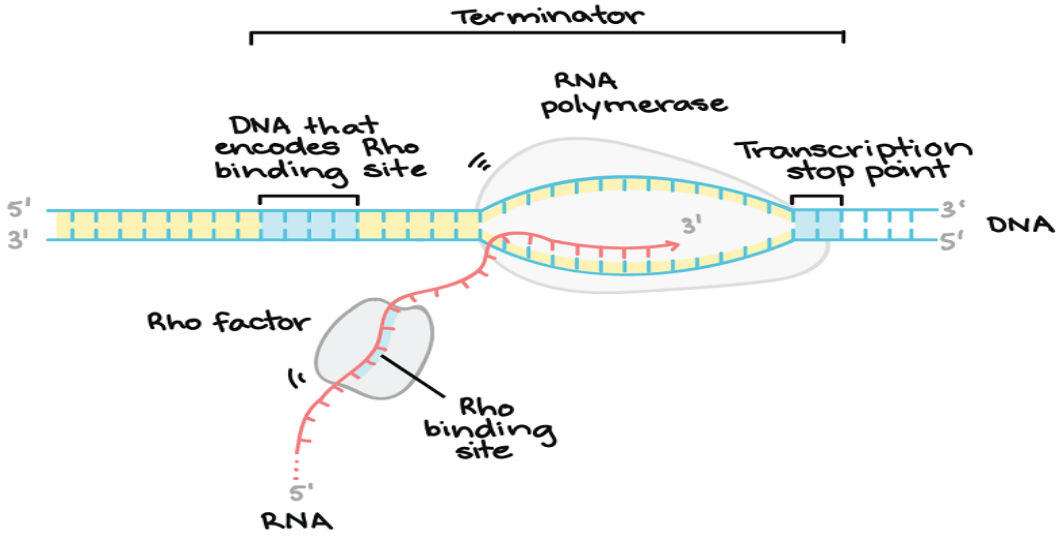
Termination

- This is the process of ending transcription, which happens when signaled by a stop sequence known as a terminator sequence.
- This happens when the RNA polymerase transcribes the terminator sequence.
- The RNA polymerase then releases the DNA temple which unwinds back to a double-helical structure.

Termination in bacteria:

There are two termination methods in bacteria

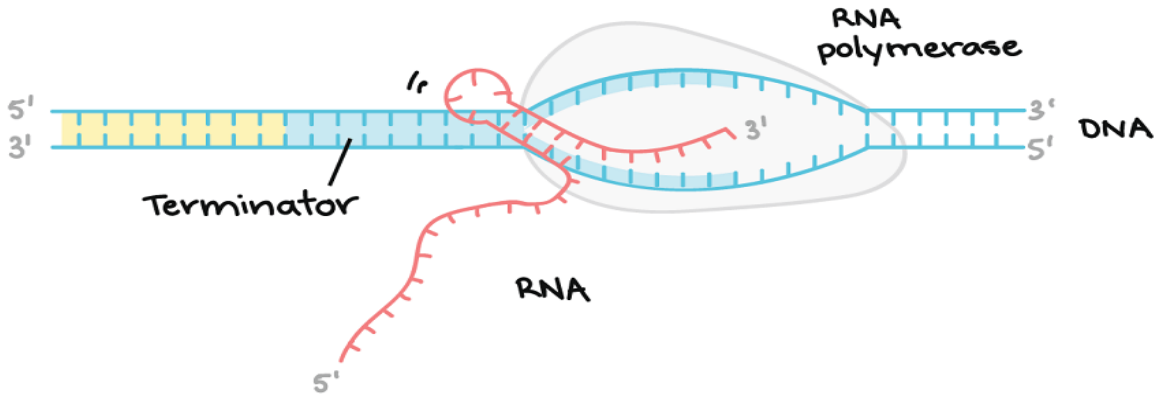
Rho-dependent termination



.

- This is the termination process where the RNA molecule contains a binding site for a protein known as the Rho factor, which binds to the DNA sequence.
- It starts to climb up the transcript towards the RNA polymerase ad reaches the transcription bubble.
- At the bubble, the Rho factor pulls the RNA transcript and the DNA template strand apart, releasing the RNA molecule and terminating the transcription process.
- A transcription stop point sequence that is found later in the DNA causes the RNA polymerase to stop and allow the Rho factor to catch up and terminate the process.

<u>Rho-independent termination</u>



- This process depends on a specific sequence found on the DNA template strand.
- During transcription, as the RNA polymerase approaches the endpoint of the gen that is transcribed, it reaches a region that is rich in Cytosine (C) and Guanine(G).

- The RNA that is transcribed from this region folds back on itself, and the complementary C and G bind together forming a stable hairpin that makes the RNA polymerase to stall.
- The hairpin is followed by a Uracil (U) in the RNA terminator which complementary to the DNA template Adenine (A).

- The U-A region forms a weak interaction with the DNA template and with the stalled RNA polymerase causes an instability allowing the enzyme to fall off and end from the new RNA transcript.

POST TRANSCRIPTIONAL MODIFICATIONS

- It may involve modifying the amino acid side chain, terminal amino or carboxyl group using covalent or enzymatic means following protein biosynthesis.
- Generally, these modifications influence the structure, stability, activity, cellular localization, or substrate specificity of the protein.
- The post-translational modification provides complexity to the proteome for diverse functions with a limited number of genes.
- Post-translational modifications (PTMs) mainly occur in the endoplasmic reticulum of the cell but sometimes continue in the Golgi bodies as well.
- After synthesis is completed, proteins can be modified by various methods such as phosphorylation, glycosylation, ADP ribosylation, hydroxylation, and addition of other groups.

1. Proteolysis

As the newly synthesized protein is released in the lumen of the ER, signal peptidases cleave peptide sequence. Apart from signal peptide, some polypeptide sequence of the protein is also cleaved resulting in the final sequence.

Example:

Insulin is synthesized in the cells in its inactive form which cannot perform its function. Post translational modifications ensure proper function which involves the removal of the part of protein to convert it into a three dimensional and fully active form.

2. Phosphorylation

Phosphoryalation is the addition of one or more phosphate groups to the protein. Post Translational Phosphorylation is one of the most common protein modifications that occur in animal cells. Majority of Phosphorylation occurs as a mechanism to regulate the biological activity of a protein. In animal cells Serine, tyrosine and thereonine are the amino acids that subjected to the Phosphorylation.

3. Glycosylation

Glycosylation is the addition of carbohydrate molecules to the polypeptide chain and modifying it into glycoproteins. Many of the proteins that are destined to become a part of plasma membrane or to be secreted from the cell, have carbohydrate chains attached to the amide nitrogen of asparagine(N linked) or the hydroxyl groups of serine, threonine(O linked). N Glycosylation occurs in ER and O Glycosylation occurs in the Golgi complex.

4. Sulfation

Sulfate modifications takes place by the addition of sulphate molecules and these modifications of proteins occurs at tyrosine residues. Tyrosine sulfation accomplished via the activity of tyrosyl protein sulfotransferases (TPST) which are membrane associated enzymes of trans-Golgi network. There are two

known TPSTs. TPST-1 TPST-2 The universal phosphate donor is 3'-phosphoadenosyl- 5'-phosphosulphate (PSPA).

5. Methylation

The transfer of one-carbon methyl groups to nitrogen or oxygen to amino acid side chains increases the hydrophobicity of the protein and can neutralize a negative amino acid charge when bound to carboxylic acids. Methylation is mediated by methyltransferases and S-adenosyl methionine (SAM) is the primary methyl group donor.

6. Hydroxylation

The biological process of addition of a hydroxy group to a protein amino acid is called Hydroxylation. Protein hydroxylation is one type of PTM that involves the conversion of –CH group into –COH group and these hydroxylated amino acids are involved in the regulation of some important factors called transcription factors. Among the 20 amino acids, the two amino acids regulated by this method are proline and lysine.

7. Others

Disulfide bond formation

Stabilizes protein structure and involved in redox processes.

Lipidylation, Acetylation, Prenylation etc.

After synthesis is completed, proteins can be modified by various methods such as Phosphorylation, Glycosylation, ADP ribosylation, hydroxylation, and addition of other groups.

RNA Synthesis Inhibitors

- RNA stands for Ribonucleic acid is a polymer of ribonucleotides.
- Transcription is the process in which DNA is transcribed into RNA with the help of the enzyme RNA polymerase. Transcription involves three steps; elongation, initiation, and termination.
- Rifamycins group of antibiotics inhibit RNA polymerase in RNA synthesis.

- Actinomycin D, an anticancer drug; a chemotherapeutic drug is also an RNA synthesis inhibitor.

Rifamycins:

- Antimicrobials having basket-like molecular structure belong to the family ansamycin.
- Rifamycins were isolated from an actinomycete which was named **Streptomyces mediterranei** which was renamed and changed into Nocardia mediterranea.
- And later the actinomycete was reclassified as Amycolatopsis mediterranei. Rifamycins group of antibiotics shows better activity against Mycobacteria and is also effective against Gram-negative bacteria.
- Rifamycin group of antibiotics are lipid-soluble which are broad-spectrum bacteriostatic first-line anti-tuberculosis drugs.
- Rifamycin B was the parent compound of the Rifamycin group of antibiotics.
- It was originated in the presence of diethylbarburitic acid. Based on production, Rifamycin is classified into natural and semi-synthetic rifamycins. Rifampin and Rifaximin are semi-synthetic rifamycins and Rifamycin CV, Rifamycin SV is natural rifamycins.
- Rifamycins have several derivatives which include rifampin, Rifamycin CV, and Rifamycin SV which have different structures and properties.
- Since resistivity is growing rapidly rifamycins group of antibiotics are used in combination with other drugs to treat mycobacterial infections.

Mechanism of action of RNA Synthesis Inhibitors

- Rifamycin including other antimicrobials of this group is considered to treat Mycobacterium as well as other Gram-positive and negative.
- It inhibits RNA synthesis. Rifamycin's mechanism of action shows the binding of antibacterial to the DNA-dependent RNA polymerase by binding to their beta-subunits (α1, β, β1, and Ϭ).
- B-subunit is the binding site for rifampicin which is encoded by the gene *rpoB*. It blocks the initiation of mRNA transcription.
- Ribosomal and transfer RNA are also equally affected as mRNA.
- And further, it prevents the translation of polypeptides.

Examples of RNA Synthesis Inhibitors

- Rifampicin
- Rifaximin
- Rifapentine

Mechanisms of resistance of RNA Synthesis Inhibitors

- Multi-drug-resistant tuberculosis is a serious issue. The resistivity in the rifamycin group is due to the mutation in the rpoB gene that decreases the efficacy to act on the infection.

- The mutation involved is a point mutation that results in the substitution of amino acid which either may be insertion or deletion.
- H406, S411, and D396 are the three amino acids that are mutated in resisted isolates are involved in oxygen and hydrogen interactions at the 8 and 21 positions.
- The other sites are susceptible to mutation. rpoB gene mutation results in lowering of affinity of enzyme which binds to the protein (wild-type).
- The lowering affinity between antimicrobials and target match up with the decreased sensitivity of the pathogens to inhibition by rifamycins.

UNIT - 4

Genetic Code

The genetic code can be defined as the set of certain rules using which the living cells translate the information encoded within genetic material (DNA or mRNA sequences). The ***ribosomes*** are responsible to accomplish the process of translation. They link the amino acids in an mRNA-specified (messenger RNA) order using tRNA (transfer RNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time.

Genetic Code Table

The complete set of relationships among ***amino acids*** and ***codons*** is said to be a genetic code which is often summarized in a table.

GENETIC CODE TABLE

FIRST LETTER	SECOND LETTER: U	SECOND LETTER: C	SECOND LETTER: A	SECOND LETTER: G	THIRD LETTER
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

It can be seen that many amino acids are shown in the table by more than one codon. For example, there are six ways to write leucine in mRNA language.

Note: A **codon** is a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule.

A key point of the genetic code is its universal nature. This indicates that virtually all species with minor exceptions use the genetic code for protein synthesis.

In other words, genetic code is defined as the nucleotide sequence of the base on DNA which is translated into a sequence of amino acids of the protein to be synthesized.

Properties of Genetic Code

- Triplet code
- Non-ambiguous and Universal
- Degenerate code
- Nonoverlapping code
- Commaless
- Start and Stop Codons
- Polarity

These properties of genetic code are explained below.

- **Triplet code**

A codon or a code word is defined as a group of bases that specify an amino acid. There is strong evidence, which proves that a sequence of three nucleotides codes for an amino acid in the protein, i.e., the code is a ***triplet***.

The four bases of nucleotide i.e, (A, G, C, and U) are used to produce three-base codons. The 64 codons involve sense codons (that specify amino acids). Hence, there are 64 codons for 20 amino acids since every codon for one amino acid means that there exist more than code for the same amino acid.

- **Commaless code**

No room for punctuation in between which indicates that every codon is adjacent to the previous one without any nucleotides between them.

- **Non overlapping code**

The code is read sequentially in a group of three and a nucleotide which becomes a part of triplet never becomes part of the next triplet.

For example

5'-UCU-3' codes for Serine

5'-AUG-3' codes for methionine

Polarity

Each triplet is read from 5' → 3' direction and the beginning base is 5' followed by the base in the middle then the last base which is 3'. This implies that the codons have a ***fixed polarity*** and if the codon is read in the reverse direction, the base sequence of the codon would reverse and would specify two different proteins.

Degenerate code

Every amino acid except tryptophan (UGG) and methionine (AUG) is coded by various codons, i.e, a few codons are synonyms and this aspect is known as the ***degeneracy of genetic code***. For instance, UGA codes for tryptophan in yeast mitochondria.

Start and Stop Codons

Generally, ***AUG codon*** is the initiating or start codon. The polypeptide chain starts either with eukaryotes (methionine) or prokaryotes (N- formylmethionine).

On the other hand, ***UAG, UAA*** and ***UGA*** are called as termination codons or stop codons. These are not read by any tRNA molecules and they never code for any amino acids.

Non-ambiguous and Universal

The genetic code is non-ambiguous which means a specific codon will only code for a particular amino acid. Also, the same genetic code is seen valid for all the organisms i.e. they are universal.

Ribosomes

"Ribosomes are one of the most important cell organelles composed of RNA and protein that converts genetic code into chains of amino acids."

A ribosome is a complex molecular machine found inside the living cells that produce proteins from amino acids during a process called protein synthesis or translation. The process of protein synthesis is a primary function, which is performed by all living cells.

Ribosomes are specialized cell organelles and are found in both prokaryotic and eukaryotic cells. Every living cell requires ribosomes for the production of proteins.

This cell organelle also functions by binding to a messenger ribonucleic acid (mRNA) and decoding the information carried by the nucleotide sequence of the mRNA. They transfer RNAs (tRNAs) comprising amino acids and enter into the ribosome at the acceptor site. Once it gets bound up, it adds amino acid to the growing protein chain on tRNA.

A ribosome is a complex of RNA and protein and is, therefore, known as a ribonucleoprotein. It is composed of two subunits – smaller and larger.

The smaller subunit is where the mRNA binds and is decoded, and in the larger subunit, the amino acids get added. Both of the subunits contain both protein and ribonucleic acid components.

The two subunits are joined to each other by interactions between the rRNAs in one subunit and proteins in the other subunit. Ribosomes are located inside the cytosol found in the plant cell and animal cells.

The ribosome structure includes the following:

- It is located in two areas of cytoplasm.
- Scattered in the cytoplasm.
- Prokaryotes have 70S ribosomes while eukaryotes have 80S ribosomes.
- Around 62% of ribosomes are comprised of RNA, while the rest is proteins.
- The structure of free and bound ribosomes is similar and is associated with protein synthesis.

Ribosomes Function

The important ribosome function includes:

1. It assembles amino acids to form proteins that are essential to carry out cellular functions.
2. The DNA produces mRNA by the process of DNA transcription.
3. The mRNA is synthesized in the nucleus and transported to the cytoplasm for the process of protein synthesis.
4. The ribosomal subunits in the cytoplasm are bound around mRNA polymers. The tRNA then synthesizes proteins.
5. Ribosomes are the site of protein synthesis.
6. The proteins synthesized in the cytoplasm are utilized in the cytoplasm itself, the proteins synthesized by bound ribosomes are transported outside the cell.

Protein Synthesis

Protein Synthesis is a process of synthesizing proteins in a chain of amino acids **known as polypeptides. It is the second part of the** central dogma **in genetics.**

- It takes place in the ribosomes found in the cytosol or those attached to the rough endoplasmic reticulum.
- The functions of the ribosome are to read the sequence of the codons in mRNA and the tRNA molecules that transfer or transport or bring the amino acids to the ribosomes in the correct sequence. However, other molecules are also involved in the process of translation such as various enzymatic factors.
- The translation process involves reading the genetic code in mRNA to make proteins.
- The entire translation process can be summarized into three phases: Initiation, elongation, and termination.

Protein Synthesis Machinery

The translation process is aided by two major factors: **A translator** – this is the molecule that conducts the translation; **substrate** – this is where the mRNA is translated into a new protein (translator desk). The translation process is guided by machinery composed of:

Ribosomes

- Ribosomes are made of ribosomal RNA (rRNA) and proteins, and therefore they are also named ribozymes because the rRNA has enzymatic activity. the rRNA has the peptidyl transferase activity that bonds the amino acids.
- The ribosomes have two subunits of rRNA and proteins, a large subunit with three active sites (E, P, A) which are critical for the catalytic activity of ribosomes.

Transfer RNA (tRNA)

Each tRNA has an anticodon for the amino acid codon it carries which are complementary to each other. For example; Lysine is coded by AAG, and therefore the anticodon that will be carried by tRNA will be UUC, therefore when the codon AAG appears, an anticodon UUC of tRNA will bind to it temporarily.

- When tRNA is bound to mRNA, the tRNA then releases its amino acid. rRNA then helps to form bonds between the amino acids as they are transported to the ribosomes one by one, thus creating a polypeptide chain. The polypeptide chain keeps growing until it reaches a stop codon.

Protein Synthesis enzymes and functions

-
-

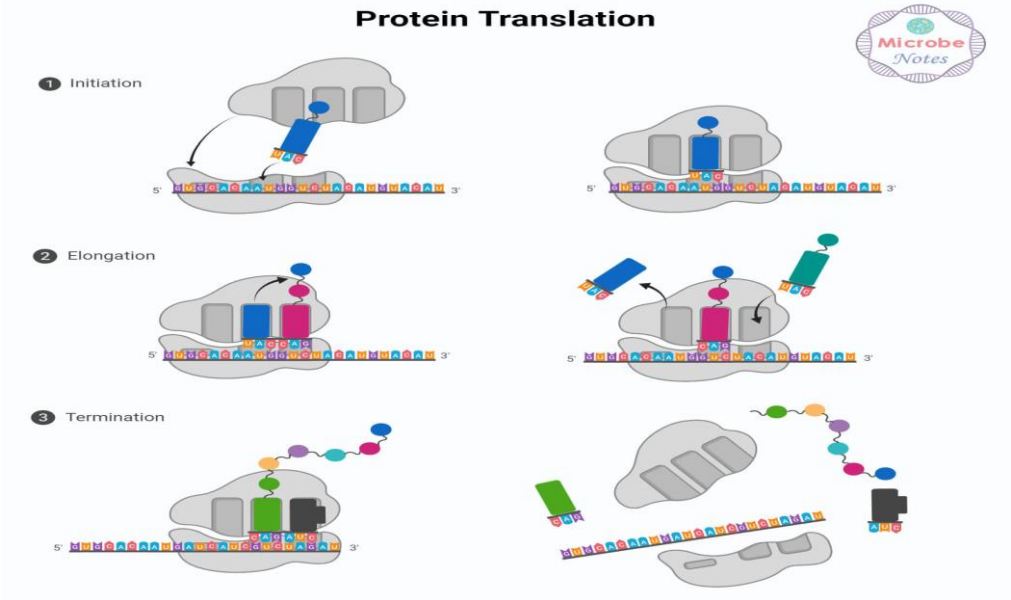
- Peptidyl transferase is the main enzyme used in Translation. It is found in the ribosomes with an enzymatic activity that catalyzes the formation of a covalent peptide bond between the adjacent amino acids.
- The enzyme's activity is to form peptide bonds between adjacent amino acids using tRNAs during translation.
- The enzyme's activity uses two substrates of which one has the growing peptide chain and the other bears the amino acid that is added to the chain.
- It is located in the large subunit of the ribosomes and therefore, the primary function of peptidyl transferase is to catalyze the addition of amino acid residues allowing the polypeptide chain to grow.
- The peptidyl transferase enzyme is entirely made up of RNA and its mechanism is mediated by ribosomal RNA (rRNA), which is a ribozyme, made up of ribonucleotides.
- In prokaryotes, the 23S subunit contains the peptidyl transferase between the A-site and the O-site of tRNA while in eukaryotes, it is found in the 28S subunit.

Protein Synthesis steps

Translation Initiation

- Protein synthesis initiation is triggered by the presence of several initiation factors IF1, IF2, and IF3, including mRNA, ribosomes, tRNA.
- The small subunit binds to the upstream on the 5′ end at the start of mRNA. The ribosome scans the mRNA in the 5′ to 3′ direction until it encounters the start codon (AUG or GUG or UUG). When either of these start codons is present, it is recognized by the initiator fMet-tRNA (N-formylMet-tRNA). This initiator factor carries the methionine (Met) which binds to the P site on the ribosome.
- This synthesizes the first amino acid polypeptide known as N-formylmethionine. The initiator fMet-tRNA has a normal methionine anticodon therefore it inserts the N-formylmethionine. This means that methionine is the first amino acid that is added and appears in the chain.
- Generally, there are three steps in the initiation process of translation;

1. Initiation of the binding of mRNA to the small ribosome subunit (the 30S), stimulating the initiator factor IF3. this dissociates the ribosomal subunits into two.
2. The initiator factor IF2 then binds to the Guanine-triphosphate (GTP) and to the initiator fMet-tRNA to the P-site of the ribosomes.
3. A ribosomal protein splits the GTP that is bound to IF2 thus helping in driving the assembly of the two ribosomal subunits. The IF3 and IF2 are released.



Translation Elongation

- The elongation of protein synthesis is aided by three protein factors i.e **EF-Tu, EF-Ts**, and **EF-G**.
- The ribosomal function is known to shift one codon at a time, catalyzing the processes that take place in its three sites.
- For every step, a charged tRNA enters the ribosomal complex and inserts the polypeptides that become one amino acid longer, while an uncharged tRNA departs. In prokaryotes, an amino acid is added at least every 0.05 seconds, which means that about 200 polypeptide amino acids are translated in 10 seconds.
- The bond created between each amino acid is derived from the Guanosine Triphosphate (GTP), which is similar to Adenosine Triphosphate (ATP).
- The three sites (A, P, E) all participate in the translation process, and the ribosome itself interacts with all the RNA types involved in translation.
- Therefore, three distinct steps are involved in translation, and these are;

1. The mediation of elongation Factor-Tu (EF-Tu) in the entry of amino-acyl-tRNAs to the A site. This entails the binding of EF-Tu to GTP, which activates the EF-Tu-GTP complex to bind to tRNA. The GTP then hydrolyses to GDP releasing an energy-giving phosphate molecule, thus driving the binding of aminoacyl-tRNA to the A site. At this point the EF-Tu is released, leaving the tRNA in the A-site.
2. Elongation factor EF-Ts then mediates the releasing of EF-Tu-GDP complex from the ribosomes and the formation of the EF-Tu-GTP.
3. During this translocation process, the polypeptide chain on the peptidyl-tRNA is transferred to the aminoacyl-tRNA on the A-site during a reaction that is catalyzed by a peptidyl transferase. The ribosomes then move one codon further along the mRNA in the 5′ to 3′ direction mediated by the elongation factor

EF-G. This step draws its energy from the splitting of GTP to GDP. Uncharged tRNA is released from the P-site, transferring newly formed peptidyl-tRNA from the A-site to the P-site.

Translation Termination

- Termination of the translation process is triggered by an encounter of any of the three stop codons (UAA, UAG, UGA). These triplet stop codons, however, are not recognized by the tRNA but by protein factors known as the **release factors, (RF1 and RF2)** found in the ribosomes.
- The RF1 recognizes the triplet UAA and UAG while RF2 recognizes UAA and UGA. A third factor also assists in catalyzing the termination process and it's known as **Release factor 3 (RF3).**
- When the peptidyl-tRNA from the elongation step arrives at the P site, the release factor of the stop codon binds to the A site. These releases the polypeptide from the P site allowing the ribosomes to dissociate into two subunits by the energy derived from GTP, leaving the mRNA.
- After many ribosomes have completed the translation process, the mRNA is degraded allowing its nucleotides to be reused in other transcription reactions.

Protein synthesis inhibitors

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. It usually refers to substances, such as antimicrobial drugs, that act at the ribosome level. The substances take advantage of the major differences between prokaryotic and eukaryotic ribosome structures which differ in their size, sequence, structure, and the ratio of protein to RNA. The differences in structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected.

Translation in prokaryotes involves the assembly of the components of the translation system which are: the two ribosomal subunits (the large 50S & small 30S subunits), the mRNA to be translated, the first aminoacyl tRNA, GTP (as a source of energy), and three initiation factors that help the assembly of the initiation complex. The ribosome has three sites: the A site, the P site, and the E site (not shown in). The A site is the point of entry for the aminoacyl tRNA. The P site is where the peptidyl tRNA is formed in the ribosome. The E site which is the exit site of the now uncharged tRNA after it gives its amino acid to the growing peptide chain.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and

ribosomal translocation), and termination. The following is a list of common antibacterial drugs and the stages which they target.

- Linezolid acts at the initiation stage, probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.
- Tetracyclines and Tigecycline (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs.
- Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing an increased rate of error in synthesis with premature termination.
- Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria.
- Macrolides, clindamycin, and aminoglycosides have evidence of inhibition of ribosomal translocation.
- Streptogramins also cause premature release of the peptide chain.

By targeting different stages of the mRNA translation, antimicrobial drugs can be changed if resistance develops to one or many of the drugs.